Technical Data Sheet

ReadyTube™ 200 MRS (de MAN, ROGOSA and SHARPE) Medium acc. ISO 15214

Ordering number: 1.46364.0006

For the isolation, enumeration and cultivation of Lactobacillus spp. from all types of materials. The medium conforms according to ISO 15214.

Mode of Action

The MRS agar contain polysorbate, acetate, magnesium and manganese, which are known to act as special growth factors for lactobacilli, as well as a rich nutrient base. As the medium exhibit a fairly low degree of selectivity, it may support growth of Pediococcus and Leuconostoc species and other secondary bacteria.

Typical Composition

Specified by ISO 15214		ReadyTube™ 200 MRS Medium	
Enzymatic Digest of Casein	10 g/l	Enzymatic Digest of Casein	10 g/l
Meat Extract	10 g/l	Meat Extract	10 g/l
Yeast Extract	4 g/l	Yeast Extract	4 g/l
Glucose	20 g/l	Glucose	20 g/l
Dipotassium Hydrogen Phosphate	2 g/l	K_2HPO_4	2 g/l
Polyoxyethylenesorbitan Monooleate (Tween®) 80	1.08 g/l	Polysorbate (Tween®) 80	1 ml/l *
Triammonium Citrate	2 g/l	Triammonium Citrate	2 g/l
Sodium Acetate	5 g/l	Sodium Acetate	5 g/l
Magnesium Sulfate Hepathydrate	0.2 g/l	Magnesium Sulfate x 7 H_2O	0.2 g/l
Manganese Sulfate Tetrahydrate	0.05 g/l	Manganese Sulfate x 4 H_2O	0.05 g/l
Agar	12-18 g/l	Agar	14 g/l
Water	1000 ml/l	Water	1000 ml/l

*1 ml Polysorbate (Tween®) 80 is equivalent to 1.08 g Polyoxyethylenesorbitan Monooleate (Tween®) 80.

Heat the bottled agar in steam or boiling water bath approx. 45 min.

Caution: avoid excessive or prolonged heating.

Cool the molten agar down to 45-50 °C in a water bath and maintain temperature until use.

Use the molten medium within 4 h of its preparation.

Application and Interpretation

The medium can be melted by placing in a boiling water bath as specified in ISO 11133. *Note: Avoid over heating the medium. Remove it from the boiling water bath once melted.* Transfer the molten medium in a thermostatically controlled water bath. Maintain temperature from 47°C to 50°C. It is recommended to use the medium as soon as possible.

If necessary, homogenize the sample material. Inoculate the MRS Agar with this material or with the original sample; it is best to use the pour-plate method.

Incubation: up to 3 days at 30 °C, if possible incubate the plates in a CO_2 enriched atmosphere in an anaerobic jar (e.g. with Merck Anaerocult® C or C mini).

Do not allow the surface of the plates to dry as this causes the acetate concentration to increase at the surface, which inhibits the growth of lactobacilli.

Determine the bacterial count. Identify the lactobacilli by the methods proposed by Sharpe (1962) and Sharpe et al. (1966). For further methods of differentiation and identification see Rogosa et al. (1953), Rogosa and Sharpe (1959) and Davis (1960).

Storage and Shelf Life

The product can be used for sampling until the expiry date if stored upright, protected from light and properly sealed at +4 °C to +12 °C.

The testing procedures as described on the CoA can be started up to the expiry date printed on the label.

Disposal

Please mind the respective regulations for the disposal of used culture medium (e.g. autoclave for 20 min at 121 °C, disinfect, incinerate etc.).

Quality Control

Function	Control strains	Incubation	Reference medium	Method of control	Expected results
Productivity	*Lactobacillus sakei* ATCC® 15521	69-75 h at 29-31 °C	Previously validated batch of MRS agar	Quantitative	Recovery ≥ 70 %, Characteristic colonies according to each species
	Lactococcus lactis ATCC® 19435				
	Pediococcus pentosaceus ATCC® 33316				
Selectivity	*Escherichia coli* ATCC® 8739	69-75 h at 29-31 °C	-	Qualitative	Total inhibition
	Escherichia coli ATCC® 25922				
	Bacillus cereus ATCC® 11778				

Please refer to the actual batch related Certificate of Analysis.

The performance test is in accordance with the current version of EN ISO 11133.

A recovery rate of 70 % is equivalent to a productivity value of 0.7.

Literature

Davis, J.G. (1960): The lactobacilli. I. Prog. in Industr. Microbiol. **2**: 3.

De man, J.D., Rogosa, M. and Sharpe, M.E. (1960): A Medium for the Cultivation of Lactobacilli. J. Appl. Bact. **23**: 130-135.

ISO 15214: 1998: Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of mesophilic lactic acid bacteria – colony-count technique at 30 °C.

ISO 11133:2014: Microbiology of food and animal feed and water – Preparation, production, storage and performance testing of culture media.

ISO 7218 AMD 1: 2013: Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations

Rogosa, M., Wiseman, R.F., Mitchell, J.A., Disraely, M.N. and Beaman, A.J. (1953): Species differentiation of oral lactobacilli from man including descriptions of *Lactobacillus salivarius* nov. spec. and *Lactobacillus cellobiosus* nov. spec. J. Bact. **65**: 681-699.

Rogosa, M. and Sharpe, M.E. (1959): An approach to the classification of the lactobacilli. J. Appl. Bact., **22**: 329-340.

Sharpe, M.E. (1962): Taxonomy of the Lactobacilli. Dairy Sci. Abstr., **24**: 109.

Sharpe, M.E., Fryer, T.F. and Smith, D.C. (1966): Identification of the Lactic Acid Bacteria. - in Gibbs, B.M. and Skinner, P.A.: Identification Methods for Microbiologists, Part A; 65-79.

Ordering Information

Product	Cat. No.	Pack size	Other pack sizes available
ReadyTube™ 200 MRS Medium ISO 15214	1.46364.0006	6 x 200 ml	
ReadyPlate™ MRS Medium ISO 15214	1.46717.0020	20 x 90 mm	100 x 90 mm
GranuCult™ MRS Agar ISO 15214	1.10660.0500	500 g	
Anaerocult® A (anaerobic jar)	1.13829.0001	10 x 1 pcs	
Anaerocult® A mini (anaerobic jar)	1.01611.0001	25 x 1 set	
Anaerobic jar 2.5L volume	1.16387.0001	1 unit	
Anaerocult® C	1.16275.0001	25 x 1 test	
Anaerocult® P	1.13807.0001	25 x 1 set	
Anaerotest®	1.15112.0001	50 strips	

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